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TGA ACA AGA GAG TGC TCA AGA AGC TGT CCA AGG ACG GCT CCA CAG AGG       48
 *  Thr Arg Glu Cys Ser Arg Ser Cys Pro Arg Thr Ala Pro Gln Arg
-41 -40                     -35                 -30

CAG GTG AGA GCA GTC ACG AGG AGG ACA CGG ATG GCG CAC GTG GCT GCG       96
Gln Val Arg Ala Val Thr Arg Arg Thr Arg Met Ala His Val Ala Ala
-25                 -20                 -15                 -10

GGG TCG ACT TTA GCC AGG CGG AAA AGG AGC GCC GGG GCT GGC AGC CAC      144
Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala Gly Ser His
                -5                   1               5

TGT CAA AAG ACC TCC CTG CGG GTA AAC TTC GAG GAC ATC GGC TGG GAC      192
Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile Gly Trp Asp
         10                  15                  20

AGC TGG ATC ATT GCA CCC AAG GAG TAT GAA GCC TAC GAG TGT AAG GGC      240
Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu Cys Lys Gly
     25                  30                  35

GGC TGC TTC TTC CCC TTG GCT GAC GAT GTG ACG CCG ACG AAA CAC GCT      288
Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr Lys His Ala
 40                  45                  50                  55

ATC GTG CAG ACC CTG GTG CAT CTC AAG TTC CCC ACA AAG GTG GGC AAG      336
Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys Val Gly Lys
                 60                  65                  70

GCC TGC TGT GTG CCC ACC AAA CTG AGC CCC ATC TCC GTC CTC TAC AAG      384
Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val Leu Tyr Lys
             75                  80                  85

GAT GAC ATG GGG GTG CCC ACC CTC AAG TAC CAT TAC GAG GGC ATG AGC      432
Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu Gly Met Ser
         90                  95                 100

GTG GCA GAG TGT GGG TGC AGG TAGTATCTGC CTGCGGG                       470
Val Ala Glu Cys Gly Cys Arg
    105                 110
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(57) Abstract

Purified BMP-9 proteins and processes for producing them are disclosed. The proteins may be used in the treatment of bone and cartilage defects and in wound healing and related tissue repair.

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BMP-9 COMPOSITIONS

The present invention relates to a novel family of purified proteins designated BMP-9 proteins and processes for obtaining them. These proteins may be used to induce bone and/or cartilage formation and in wound healing and tissue repair.

The murine BMP-9 DNA sequence (SEQ ID NO: 1) and amino acid sequence (SEQ ID NO: 2) are set forth in Figure 1. Human BMP-9 sequence is set forth in Figure 3 (SEQ ID NO: 8 and SEQ ID NO: 9). It is contemplated that BMP-9 proteins are capable of inducing the formation of cartilage and/or bone. BMP-9 proteins may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described below.

Murine BMP-9 is characterized by comprising amino acid #319 to #428 of Figure 1 (SEQ ID NO: 2 amino acids #1-110). Murine BMP-9 may be produced by culturing a cell transformed with a DNA sequence comprising nucleotide #610 to nucleotide #1893 as shown in Figure 1 (SEQ ID NO: 1) and recovering and purifying from the culture medium a protein characterized by the amino acid sequence comprising amino acid #319 to #428 as shown in Figure 1 (SEQ ID NO: 2) substantially free from other proteinaceous materials with which it is co-produced.

Human BMP-9 is expected to be homologous to murine BMP-9 and is characterized by comprising amino acid #1 (Ser, Ala, Gly) to #110 of Figure 3 (SEQ ID NO: 9) (Arg). The invention includes methods for obtaining the DNA sequences encoding human BMP-9. This method entails utilizing the murine BMP-9 nucleotide sequence or portions thereof to design probes to screen libraries for the human gene or fragments thereof using standard techniques. Human BMP-9 may be produced by culturing

a cell transformed with the BMP-9 DNA sequence and recovering and purifying BMP-9 from the culture medium. The expressed protein is isolated, recovered, and purified from the culture medium. The purified expressed protein is substantially free from other proteinaceous materials with which it is co-produced, as well as from other contaminants. The recovered purified protein is contemplated to exhibit cartilage and/or bone formation activity. The proteins of the invention may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described below.

Human BMP-9 may be produced by culturing a cell transformed with a DNA sequence comprising nucleotide #124 to #453 as shown in SEQ ID NO: 8 and recovering and purifying from the culture medium a protein characterized by the amino acid sequence of SEQ ID NO: 9 from amino acid #1 to amino acid #110 substantially free from other proteinaceous materials with which it is co-produced.

Another aspect of the invention provides pharmaceutical compositions containing a therapeutically effective amount of a BMP-9 protein in a pharmaceutically acceptable vehicle or carrier. BMP-9 compositions of the invention may be used in the formation of cartilage. These compositions may further be utilized for the formation of bone. BMP-9 compositions may also be used for wound healing and tissue repair. Compositions of the invention may further include at least one other therapeutically useful agent such as the BMP proteins BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, and BMP-7 disclosed for instance in PCT publications WO88/00205, WO89/10409, and WO90/11366, and BMP-8, disclosed in U.S. application Ser. No. 07/641,204 filed January 15, 1991, Ser. No. 07/525,357 filed May 16, 1990, and Ser. No. 07/800,364 filed November 20, 1991.

The compositions of the invention may comprise, in addition to a BMP-9 protein, other therapeutically useful agents including growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth

factor (TGF-α and TGF-β), and insulin-like growth factor (IGF). The compositions may also include an appropriate matrix for instance, for supporting the composition and providing a surface for bone and/or cartilage growth. The matrix may provide slow release of the osteoinductive protein and/or the appropriate environment for presentation thereof.

The BMP-9 compositions may be employed in methods for treating a number of bone and/or cartilage defects, periodontal disease and various types of wounds. These methods, according to the invention, entail administering to a patient needing such bone and/or cartilage formation wound healing or tissue repair, an effective amount of a BMP-9 protein. These methods may also entail the administration of a protein of the invention in conjunction with at least one of the novel BMP proteins disclosed in the co-owned applications described above. In addition, these methods may also include the administration of a BMP-9 protein with other growth factors including EGF, FGF, TGF-α, TGF-β, and IGF.

Still a further aspect of the invention are DNA sequences coding for expression of a BMP-9 protein. Such sequences include the sequence of nucleotides in a 5′ to 3′ direction illustrated in Figure 1 (SEQ ID NO: 1) and Figure 3 (SEQ ID NO: 8) or DNA sequences which hybridize under stringent conditions with the DNA sequences of Figure 1 or 3 and encode a protein having the ability to induce the formation of cartilage and/or bone. Finally, allelic or other variations of the sequences of Figure 1 or 3, whether such nucleotide changes result in changes in the peptide sequence or not, are also included in the present invention.

A further aspect of the invention includes vectors comprising a DNA sequence as described above in operative association with an expression control sequence therefor. These vectors may be employed in a novel process for producing a BMP-9 protein of the invention in which a cell line transformed with a DNA sequence encoding a BMP-9 protein in

operative association with an expression control sequence therefor, is cultured in a suitable culture medium and a BMP-9 protein is recovered and purified therefrom. This process may employ a number of known cells both prokaryotic and eukaryotic as host cells for expression of the polypeptide.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description and preferred embodiments thereof.

Brief Description of the Drawing

FIG. 1 comprises DNA sequence and derived amino acid sequence of murine BMP-9 from clone ML14a further described below.

FIG. 2 comprises DNA sequence and derived amino acid sequence of human BMP-4 from lambda U2OS-3 ATCC #40342.

FIG. 3 comprises DNA sequence and derived amino acid sequence of human BMP-9 from λ FIX/H6111 ATCC # 75252.

Detailed Descripton of the Invention

The murine BMP-9 nucleotide sequence (SEQ ID NO: 1) and encoded amino acid sequence (SEQ ID NO: 2) are depicted in Figure 1. Purified murine BMP-9 proteins of the present invention are produced by culturing a host cell transformed wth a DNA sequence comprising the DNA coding sequence of Figure 1 (SEQ ID NO: 1) from nucleotide #610 to nucleotide #1893 and recovering and purifying from the culture medium a protein which contains the amino acid sequence or a substantially homologous sequence as represented by amino acid #319 to #428 of Figure 1 (SEQ ID NO: 2). The BMP-9 proteins recovered from the culture medium are purified by isolating them from other proteinaceous materials from which they are co-produced and from other contaminants present.

Human BMP-9 nucleotide and amino acid sequence is depicted in SEQ ID No: 8 and 9. Mature human BMP-9 is expected to comprise amino acid #1 (Ser, Ala, Gly) to #110 (Arg).

Human BMP-9 may be produced by culturing a cell transformed with a DNA sequence comprising nucleotide #124 to #453 as shown in SEQ ID NO: 8 and recovering and purifying from the culture medium a protein characterized by the amino acid sequence of SEQ ID NO: 9 from amino acid #1 to amino acid #110 substantially free from other proteinaceous materials with which it is co-produced.

BMP-9 proteins may be characterized by the ability to induce the formation of cartilage. BMP-9 proteins may be further characterized by the ability to induce the formation of bone. BMP-9 proteins may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described below.

The BMP-9 proteins provided herein also include factors encoded by the sequences similar to those of Figure 1 and 3 (SEQ ID NO's: 1 and 8), but into which modifications are naturally provided (e.g. allelic variations in the nucleotide sequence which may result in amino acid changes in the polypeptide) or deliberately engineered. For example, synthetic polypeptides may wholly or partially duplicate continuous sequences of the amino acid residues of Figure 1 of Figure 3 (SEQ ID NO's: 2 and 9). These sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with bone growth factor polypeptides of Figure 1 and Figure 3 may possess bone growth factor biological properties in common therewith. Thus, they may be employed as biologically active substitutes for naturally-occurring BMP-9 and other BMP-9 polypeptides in therapeutic processes.

Other specific mutations of the sequences of BMP-9 proteins described herein involve modifications of glycosylation sites. These modifications may involve O-linked

or N-linked glycosylation sites. For instance, the absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at asparagine-linked glycosylation recognition sites. The asparagine-linked glycosylation recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence.

The present invention also encompasses the novel DNA sequences, free of association with DNA sequences encoding other proteinaceous materials, and coding on expression for BMP-9 proteins. These DNA sequences include those depicted in Figure 1 or Figure 3 (SEQ ID NO's: 1 and 8) in a 5' to 3' direction and those sequences which hybridize thereto under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] and encode a protein having cartilage and/or bone inducing activity.

Similarly, DNA sequences which code for BMP-9 proteins coded for by the sequences of Figure 1 or Figure 3, but which differ in codon sequence due to the degeneracies of the genetic code or allelic variations (naturally-occurring base changes in the species population which may or may not result in an amino acid change) also encode the novel factors described herein. Variations in the DNA sequences of Figure 1 or Figure 3 (SEQ ID NO: 1 and 8) which are caused by point mutations or by induced modifications (including insertion, deletion, and substitution) to enhance the activity, half-life or production of the polypeptides encoded are also encompassed in the invention.

Another aspect of the present invention provides a novel

method for producing BMP-9 proteins. The method of the present invention involves culturing a suitable cell line, which has been transformed with a DNA sequence encoding a BMP-9 protein of the invention, under the control of known regulatory sequences. The transformed host cells are cultured and the BMP-9 proteins recovered and purified from the culture medium. The purified proteins are substantially free from other proteins with which they are co-produced as well as from other contaminants.

Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening, product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U.S. Patent 4,419,446. Another suitable mammalian cell line, which is described in the accompanying examples, is the monkey COS-1 cell line. The mammalian cell CV-1 may also be suitable.

Bacterial cells may also be suitable hosts. For example, the various strains of E. coli (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Pseudomonas, other bacilli and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art may also be available as host cells for expression of the polypeptides of the present invention. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g. Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein.

Another aspect of the present invention provides vectors for use in the method of expression of these novel BMP-9 polypeptides. Preferably the vectors contain the full novel

DNA sequences described above which encode the novel factors of the invention. Additionally the vectors also contain appropriate expression control sequences permitting expression of the BMP-9 protein sequences. Alternatively, vectors incorporating modified sequences as described above are also embodiments of the present invention. The vectors may be employed in the method of transforming cell lines and contain selected regulatory sequences in operative association with the DNA coding sequences of the invention which are capable of directing the replication and expression thereof in selected host cells. Regulatory sequences for such vectors are known to those skilled in the art and may be selected depending upon the host cells. Such selection is routine and does not form part of the present invention.

A protein of the present invention, which induces cartilage and/or bone formation in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage defects in humans and other animals. Such a preparation employing a BMP-9 protein may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery. A BMP-9 protein may be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. BMP-9 polypeptides of the invention may also be useful in the treatment of osteoporosis. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g. European patent applications 148;155 and 169,016 for discussions thereof.

The proteins of the invention may also be used in wound healing and related tissue repair. The types of wounds include, but are not limited to burns, incisions and ulcers. (See, e.g. PCT Publication WO84/01106 for discussion of wound healing and related tissue repair).

It is further contemplated that proteins of the invention may increase neuronal survival and therefore be useful in transplantation and treatment of conditions exhibiting a decrease in neuronal survival.

A further aspect of the invention is a therapeutic method and composition for repairing fractures and other conditions related to cartilage and/or bone defects or periodontal diseases. The invention further comprises therapeutic methods and compositions for wound healing and tissue repair. Such compositions comprise a therapeutically effective amount of at least one of the BMP-9 proteins of the invention in admixture with a pharmaceutically acceptable vehicle, carrier or matrix.

It is expected that the proteins of the invention may act in concert with or perhaps synergistically with other related proteins and growth factors. Further therapeutic methods and compositions of the invention therefore comprise a therapeutic amount of at least one BMP-9 protein of the invention with a therapeutic amount of at least one of the other BMP proteins disclosed in co-owned applications described above. Such combinations may comprise separate molecules of the BMP proteins or heteromolecules comprised of different BMP moieties. For example, a method and composition of the invention may comprise a disulfide linked dimer comprising a BMP-9 protein subunit and a subunit from one of the "BMP" proteins described above. A further embodiment may comprise a heterodimer of BMP-9 moieties. Further, BMP-9 proteins may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF),

transforming growth factors (TGF-α and TGF-β), and insulin-like growth factor (IGF).

The preparation and formulation of such physiologically acceptable protein compositions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art. The therapeutic compositions are also presently valuable for veterinary applications due to the lack of species specificity in BMP proteins. Particularly domestic animals and thoroughbred horses in addition to humans are desired patients for such treatment with BMP-9 of the present invention.

The therapeutic method includes administering the composition topically, systemically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than the BMP-9 proteins which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the BMP composition in the methods of the invention.

Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering BMP-9 or other BMP proteins to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. The matrix may provide slow release of BMP-9 and/or the appropriate environment for presentation thereof. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular

application of the BMP-9 compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of the BMP-9 protein, e.g. amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the size of a wound, type of damaged tissue, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the types of BMP proteins in the composition. The addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of bone growth and/or repair, for example, x-rays, histomorphometric determinations and tetracycline labeling.

The following examples illustrate practice of the present invention in recovering and characterizing murine BMP-9 protein and employing it to recover the human and other BMP-9 proteins, obtaining the human proteins and expressing the proteins via recombinant techniques.

EXAMPLE I

Murine BMP-9

750,000 recombinants of a mouse liver cDNA library made in the vector lambdaZAP (Stratagene/Catalog #935302) are plated and duplicate nitrocellulose replicas made. A fragment of human BMP-4 DNA corresponding to nucleotides 1330-1627 of Figure 2 (SEQ ID NO: 3) (the human BMP-4 sequence) is ^{32}P-labeled by the random priming procedure of Feinberg et al. [Anal. Biochem. 132: 6-13 (1983)] and hybridized to both sets of filters in SHB at 60°C for 2 to 3 days. Both sets of filters are washed under reduced stringency conditions (4X SSC, 0.1% SDS at 60°C). Many duplicate hybridizing recombinants of various intensities (approximately 92) are noted. 50 of the strongest hybridizing recombinant bacteriophage are plaque purified and their inserts are transferred to the plasmid Bluescript SK (+/-) according to the in vivo excision protocol described by the manufacturer (Stratagene). DNA sequence analysis of several recombinants indicate that they encode a protein homologous to other BMP proteins and other proteins in the TGF-β family. The DNA sequence and derived amino acid sequence of one recombinant, designated ML14a, is set forth in Figure 1. (SEQ ID NO: 1)

The nucleotide sequence of clone ML14a contains an open reading frame of 1284 bp, encoding a BMP-9 protein of 428 amino acids. The encoded 428 amino acid BMP-9 protein is contemplated to be the primary translation product as the coding sequence is preceded by 609 bp of 5' untranslated sequence with stop codons in all three reading frames. The 428 amino acid sequence predicts a BMP-9 protein with a molecular weight of 48,000 daltons.

Based on knowledge of other BMP proteins and other proteins within the TGF-β family, it is predicted that the precursor polypeptide would be cleaved at the multibasic sequence ARG-ARG-LYS-ARG in agreement with a proposed consensus

proteolytic processing sequence of ARG-X-X-ARG. Cleavage of the BMP-9 precursor polypeptide at this location would generate a 110 amino acid mature peptide beginning with the amino acid SER at position #319. The processing of BMP-9 into the mature form is expected to involve dimerization and removal of the N-terminal region in a manner analogous to the processing of the related protein TGF-β [L.E. Gentry, et al., Molec. & Cell. Biol. 8:4162 (1988); R. Derynck, et al., Nature 316:701 (1985)].

It is contemplated therefore that the mature active species of murine BMP-9 comprises a homodimer of 2 polypeptide subunits, each subunit comprising amino acids #319-#428 with a predicted molecular weight of approximately 12,000 daltons. Further active species are contemplated comprising amino acids #326 - #428 thereby including the first conserved cysteine residue. As with other members of the BMP and TGF-β family of proteins, the carboxy-terminal region of the BMP-9 protein exhibits greater sequence conservation than the more amino-terminal portion. The percent amino acid identity of the murine BMP-9 protein in the cysteine-rich C-terminal domain (amino acids #326 - #428) to the corresponding region of other human BMP proteins and other proteins within the TGF-β family is as follows: BMP-2, 53%; BMP-3, 43%; BMP-4, 53%; BMP-5, 55%; BMP-6, 55%; BMP-7, 53%; Vgl, 50%; GDF-1, 43%; TGF-β1, 32%; TGF-β2, 34%; TGF-β3, 34%; inhibin β(B), 34%; and inhibin β(A), 42%.

EXAMPLE II

Human BMP-9

Murine and human osteoinductive factor genes are presumed to be significantly homologous, therefore the murine coding sequence or a portion thereof is used as a probe to screen a human genomic library or as a probe to identify a human cell line or tissue which synthesizes the analogous human cartilage and/or bone protein. A human genomic library (Toole et al., supra) may be screened with such a probe, and presumptive

positives isolated and DNA sequence obtained. Evidence that this recombinant encodes a portion of the human BMP-9 relies of the murine/human protein and gene structure homologies.

Once a recombinant bacteriophage containing DNA encoding portion of the human cartilage and/or bone inductive factor molecule is obtained, the human coding sequence can be used as a probe to identify a human cell line or tissue which synthesizes BMP-9. Alternatively, the murine coding sequence can be used as a probe to identify such human cell line or tissue. Briefly described, RNA is extracted from a selected cell or tissue source and either electrophoresed on a formaldehyde agarose gel and transferred to nitrocellulose, or reacted with formaldehyde and spotted on nitrocellulose directly. The nitrocellulose is then hybridized to a probe derived from a coding sequence of the murine or human BMP-9. mRNA is selected by oligo (dT) cellulose chromatography and cDNA is synthesized and cloned in lambda gt10 or lambda ZAP by established techniques (Toole et al., <u>supra</u>).

Additional methods known to those skilled in the art may be used to isolate the human and other species' BMP-9 proteins of the invention.

A. <u>Isolation of Human BMP-9 DNA</u>

One million recombinants of a human genomic library constructed in the vector λFIX (Stratagene catalog # 944201) are plated and duplicate nitrocellulose replicas made. Two oligonucleotides probes designed on the basis of nucleotides #1665-#1704 and #1837-#1876 of the sequence set forth in Figure 1 (SEQ ID NO:1) are synthesized on an automated DNA synthesizer. The sequence of these two oligonucleotides is indicated below:

#1: CTATGAGTGTAAAGGGGGTTGCTTCTTCCCATTGGCTGAT

#2: GTGCCAACCCTCAAGTACCACTATGAGGGGATGAGTGTGG

These two oligonucleotide probes are radioactively labeled with

γ^{32}P-ATP and each is hybridized to one set of the duplicate nitrocellulose replicas in SHB at 65°C and washed with 1X SSC, 0.1% SDS at 65°C. Three recombinants which hybridize to both oligonucleotide probes are noted. All three positively hybridizing recombinants are plaque purified, bacteriophage plate stocks are prepared and bacteriophage DNA is isolated from each. The oligonucleotide hybridizing regions of one of these recombinants, designated HG111, is localized to a 1.2 kb Pst I/Xba I fragment. This fragment is subcloned into a plasmid vector (pGEM-3) and DNA sequence analysis is performed. HG111 was deposited with the ATCC, 12301 Parklawn Drive, Rockville, Maryland USA on June 16, 1992 under the requirements of the Budapest Treaty and designated as ATCC # 75252. This subclone is designated pGEM-111. A portion of the DNA sequence of clone pGEM-111 is set forth in Figure 3 (SEQ ID NO:8/ HUMAN BMP-9 sequence). This sequence encodes the entire mature region of human BMP-9 and a portion of the propeptide. It should be noted that this sequence consists of preliminary data. Particularly, the propeptide region is subject to further analysis and characterization. For example, nucleotides #1 through #3 (TGA) encode a translational stop which may be incorrect due to the preliminary nature of the sequence. It is predicted that additional sequences present in both pGEM-111 (the 1.2 kb PstI/XbaI fragment of HG111 subcloned into pGEM) and HG111 encode additional amino acids of the human BMP-9 propeptide region. Based on knowledge of other BMPs and other proteins within the TGF-β family, it is predicted that the precursor polypeptide would be cleaved at the multibasic sequence ARG-ARG-LYS-ARG (amino acids # -4 through # -1 of SEQUENCE ID NO:9) in agreement with a proposed consensus proteolytic processing sequence ARG-X-X-ARG. Cleavage of the human BMP-9 precursor polypeptide at this location would generate a 110 amino acid mature peptide beginning with the amino acid SER at position #1 of SEQUENCE ID NO:9 (encoded by

nucleotides #124 through #126 of SEQUENCE ID NO:8). The processing of human BMP-9 into the mature form is expected to involve dimerization and removal of the N-terminal region in a manner analogous to the processing of the related protein TGF-β [L.E. Gentry, et al., Molec. & Cell. Biol. 8:4162 (1988); R. Derynck, et al., Nature 316:701 (1985)].

It is contemplated therefore that the mature active species of human BMP-9 comprises a homodimer of two polypeptide subunits, each subunit comprising amino acids #1 through #110 of SEQUENCE ID NO:9, with a predicted molecular weight of 12,000 daltons. Further active species are contemplated comprising amino acids #8 through #110 thereby including the first conserved cysteine residue. As with other members of the BMP and TGF-β family of proteins, the carboxy-terminal portion of the human BMP-9 sequence exhibits greater sequence conservation than the amino-terminal portion. the percent amino acid identity of the human BMP-9 protein in the cysteine-rich C-terminal domain (amino acids #8 through #110) to the corresponding region of other human BMP proteins and other proteins within the TGF-β family is as follows: BMP-2, 52%; BMP-3, 40%; BMP-4, 52%; BMP-5, 55%; BMP-6, 55%; BMP-7, 53%; murine BMP-9, 97%; Vg1, 50%; GDF-1, 44%; TGF-β1, 32%; TGF-β2, 32%; TGF-β3, 32%; inhibin β (B), 35%; and inhibin β (A), 41%.

EXAMPLE III

Rosen Modified Sampath-Reddi Assay

A modified version of the rat bone formation assay described in Sampath and Reddi, Proc. Natl. Acad. Sci. U.S.A., 80:6591-6595 (1983) is used to evaluate bone and/or cartilage activity of the BMP proteins. This modified assay is herein called the Rosen-modified Sampath-Reddi assay. The ethanol precipitation step of the Sampath-Reddi procedure is replaced by dialyzing (if the composition is a solution) or diafiltering (if the composition is a suspension) the fraction to be assayed against water. The solution or suspension is then redissolved

in 0.1 % TFA, and the resulting solution added to 20mg of rat matrix. A mock rat matrix sample not treated with the protein serves as a control. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted subcutaneously in the abdominal thoracic area of 21 - 49 day old male Long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi et al., Proc. Natl Acad Sci., 69:1601 (1972)].

The other half of each implant is fixed and processed for histological analysis. 1μm glycolmethacrylate sections are stained with Von Kossa and acid fuschin to score the amount of induced bone and cartilage formation present in each implant. The terms +1 through +5 represent the area of each histological section of an implant occupied by new bone and/or cartilage cells and matrix. A score of +5 indicates that greater than 50% of the implant is new bone and/or cartilage produced as a direct result of protein in the implant. A score of +4, +3, +2 and +1 would indicate that greater than 40%, 30%, 20% and 10% respectively of the implant contains new cartilage and/or bone. In a modified scoring method, three non-adjacent sections are evaluated from each implant and averaged. "+/-" indicates tentative identification of cartilage or bone; "+1" indicates >10% of each section being new cartilage or bone; "+2", >25%; "+3", >50%; "+4", ~75%; "+5", >80%. A "-" indicates that the implant is not recovered.

It is contemplated that the dose response nature of the BMP-9 containing samples of the matrix samples will demonstrate that the amount of bone and/or cartilage formed increases with the amount of BMP-9 in the sample. It is contemplated that the control samples will not result in any bone and/or cartilage formation.

As with other cartilage and/or bone inductive proteins such as the above-mentioned "BMP" proteins, the bone and/or cartilage formed is expected to be physically confined to the

space occupied by the matrix. Samples are also analyzed by SDS gel electrophoresis and isoelectric focusing followed by autoradiography. The activity is correlated with the protein bands and pI. To estimate the purity of the protein in a particular fraction an extinction coefficient of 1 OD/mg-cm is used as an estimate for protein and the protein is run on SDS PAGE followed by silver staining or radioiodination and autoradiography.

EXAMPLE IV

Expression of BMP-9

In order to produce murine, human or other mammalian BMP-9 proteins, the DNA encoding it is transferred into an appropriate expression vector and introduced into mammalian cells or other preferred eukaryotic or prokaryotic hosts by conventional genetic engineering techniques. The preferred expression system for biologically active recombinant human BMP-9 is contemplated to be stably transformed mammalian cells.

One skilled in the art can construct mammalian expression vectors by employing the sequence of Figure 1 (SEQ ID NO: 1) or Figure 3 (SEQ ID NO: 8), or other DNA sequences encoding BMP-9 proteins or other modified sequences and known vectors, such as pCD [Okayama et al., Mol. Cell Biol., 2:161-170 (1982)], pJL3, pJL4 [Gough et al., EMBO J., 4:645-653 (1985)] and pMT2 CXM.

The mammalian expression vector pMT2 CXM is a derivative of p91023 (b) (Wong et al., Science 228:810-815, 1985) differing from the latter in that it contains the ampicillin resistance gene in place of the tetracycline resistance gene and further contains a XhoI site for insertion of cDNA clones. The functional elements of pMT2 CXM have been described (Kaufman, R.J., 1985, Proc. Natl. Acad. Sci. USA 82:689-693) and include the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5′ splice site and the majority of

the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3' splice acceptor site, a DHFR insert, the SV40 early polyadenylation site (SV40), and pBR322 sequences needed for propagation in E. coli.

Plasmid pMT2 CXM is obtained by EcoRI digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC 67122. EcoRI digestion excises the cDNA insert present in pMT2-VWF, yielding pMT2 in linear form which can be ligated and used to transform E. coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods. pMT2 CXM is then constructed using loopout/in mutagenesis [Morinaga, et al., Biotechnology 84: 636 (1984). This removes bases 1075 to 1145 relative to the Hind III site near the SV40 origin of replication and enhancer sequences of pMT2. In addition it inserts the following sequence:

5' PO-CATGGGCAGCTCGAG-3' (SEQ ID NO: 5)

at nucleotide 1145. This sequence contains the recognition site for the restriction endonuclease Xho I. A derivative of pMT2CXM, termed pMT23, contains recognition sites for the restriction endonucleases PstI, Eco RI, SalI and XhoI. Plasmid pMT2 CXM and pMT23 DNA may be prepared by conventional methods.

pEMC2b1 derived from pMT21 may also be suitable in practice of the invention. pMT21 is derived from pMT2 which is derived from pMT2-VWF. As described above EcoRI digestion excises the cDNA insert present in pMT-VWF, yielding pMT2 in linear form which can be ligated and used to transform E. Coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods.

pMT21 is derived from pMT2 through the following two modifications. First, 76 bp of the 5' untranslated region of the DHFR cDNA including a stretch of 19 G residues from G/C tailing for cDNA cloning is deleted. In this process, a XhoI site is inserted to obtain the following sequence immediately

upstream from DHFR: 5′ -CTGCAGGCGAGCCTGAATTCCTCGAGCCATCATG-3′
PstI Eco RI XhoI
(SEQ ID NO: 6)

Second, a unique ClaI site is introduced by digestion with EcoRV and XbaI, treatment with Klenow fragment of DNA polymerase I, and ligation to a ClaI linker (CATCGATG). This deletes a 250 bp segment from the adenovirus associated RNA (VAI) region but does not interfere with VAI RNA gene expression or function. pMT21 is digested with EcoRI and XhoI, and used to derive the vector pEMC2B1.

A portion of the EMCV leader is obtained from pMT2-ECAT1 [S.K. Jung, et al, J. Virol 63:1651-1660 (1989)] by digestion with Eco RI and PstI, resulting in a 2752 bp fragment. This fragment is digested with TaqI yielding an Eco RI-TaqI fragment of 508 bp which is purified by electrophoresis on low melting agarose gel. A 68 bp adapter and its complementary strand are synthesized with a 5′ TaqI protruding end and a 3′ XhoI protruding end which has the following sequence:

5′-CGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTT
TaqI

GAAAAACACGATTGC-3′
XhoI (SEQ ID NO: 7)

This sequence matches the EMC virus leader sequence from nucleotide 763 to 827. It also changes the ATG at position 10 within the EMC virus leader to an ATT and is followed by a XhoI site. A three way ligation of the pMT21 Eco RI-XhoI fragment, the EMC virus EcoRI-TaqI fragment, and the 68 bp oligonucleotide adapter TaqI-XhoI adapter resulting in the vector pEMC2β1.

This vector contains the SV40 origin of replication and enhancer, the adenovirus major late promoter, a cDNA copy of the majority of the adenovirus tripartite leader sequence, a small hybrid intervening sequence, an SV40 polyadenylation

signal and the adenovirus VA I gene, DHFR and β-lactamase markers and an EMC sequence, in appropriate relationships to direct the high level expression of the desired cDNA in mammalian cells.

The construction of vectors may involve modification of the BMP-9 DNA sequences. For instance, BMP-9 cDNA can be modified by removing the non-coding nucleotides on the 5′ and 3′ ends of the coding region. The deleted non-coding nucleotides may or may not be replaced by other sequences known to be beneficial for expression. These vectors are transformed into appropriate host cells for expression of BMP-9 proteins.

One skilled in the art can manipulate the sequences of Figure 1 or Figure 3 (SEQ ID NO: 1 and 8) by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with bacterial sequences to create bacterial vectors for intracellular or extracellular expression by bacterial cells. For example, the coding sequences could be further manipulated (e.g. ligated to other known linkers or modified by deleting non-coding sequences therefrom or altering nucleotides therein by other known techniques). The modified BMP-9 coding sequence could then be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al., <u>Proc. Natl Acad. Sci. USA</u>, 77:5230-5233 (1980). This exemplary bacterial vector could then be transformed into bacterial host cells and a BMP-9 protein expressed thereby. For a strategy for producing extracellular expression of BMP-9 proteins in bacterial cells, see, e.g. European patent application EPA 177,343.

Similar manipulations can be performed for the construction of an insect vector [See, e.g. procedures described in published European patent application 155,476] for expression in insect cells. A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of the factors of the present invention by yeast cells. [See, e.g., procedures

described in published PCT application WO86/00639 and European patent application EPA 123,289].

A method for producing high levels of a BMP-9 protein of the invention in mammalian cells may involve the construction of cells containing multiple copies of the heterologous BMP-9 gene. The heterologous gene is linked to an amplifiable marker, e.g. the dihydrofolate reductase (DHFR) gene for which cells containing increased gene copies can be selected for propagation in increasing concentrations of methotrexate (MTX) according to the procedures of Kaufman and Sharp, J. Mol. Biol., 159:601-629 (1982). This approach can be employed with a number of different cell types.

For example, a plasmid containing a DNA sequence for a BMP-9 of the invention in operative association with other plasmid sequences enabling expression thereof and the DHFR expression plasmid pAdA26SV(A)3 [Kaufman and Sharp, Mol. Cell. Biol., 2:1304 (1982)] can be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by various methods including calcium phosphate coprecipitation and transfection, electroporation or protoplast fusion. DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum, and subsequently selected for amplification by growth in increasing concentrations of MTX (e.g. sequential steps in 0.02, 0.2, 1.0 and 5uM MTX) as described in Kaufman et al., Mol Cell Biol., 5:1750 (1983). Transformants are cloned, and biologically active BMP-9 expression is monitored by the Rosen-modified Sampath - Reddi rat bone formation assay described above in Example III. BMP-9 expression should increase with increasing levels of MTX resistance. BMP-9 polypeptides are characterized using standard techniques known in the art such as pulse labeling with [35S] methionine or cysteine and polyacrylamide gel electrophoresis. Similar procedures can be followed to produce other related BMP-9 proteins.

A. BMP-9 Vector Construction

In order to produce human BMP-9 proteins of the invention DNA sequences encoding the mature region of the human BMP-9 protein may be joined to DNA sequences encoding the propeptide region of the murine BMP-9 protein. This murine/human hybrid DNA sequence is inserted into an appropriate expression vector and introduced into mammalian cells or other preferred eukaryotic or prokaryotic hosts by conventional genetic engineering techniques. The construction of this murine/human BMP-9 containing expression plasmid is described below.

A derivative of the human BMP-9 sequence (SEQ ID NO:8) comprising the nucleotide sequence from nucleotide #105 to #470 is specifically amplified. The following oligonucleotides are utilized as primers to allow the amplification of nucleotides #105 to #470 of the human BMP-9 sequence (SEQ ID NO:8) from clone pGEM-111 described above.

#3 ATCGGGCCCCTTTTAGCCAGGCGGAAAAGGAG

#4 AGCGAATTCCCCGCAGGCAGATACTACCTG

This procedure generates the insertion of the nucleotide sequence ATCGGGCCCCT immediately preceeding nucleotide #105 and the insertion of the nucleotide sequence GAATTCGCT immediately following nucleotide #470. The addition of these sequences results in the creation of an Apa I and EcoR I restriction endonuclease site at the respective ends of the specifically amplified DNA fragment. The resulting 374 bp Apa I/EcoR I fragment is subcloned into the plasmid vector pGEM-7Zf(+) (Promega catalog# p2251) which has been digested with Apa I and EcoR I. The resulting clone is designated phBMP9mex-1.

The following oligonucleotides are designed on the basis of murine BMP-9 sequences (SEQ ID NO:1) and are modified to facilitate the construction of the murine/human expression plasmid referred to above:

#5

GATTCCGTCGACCACCATGTCCCCTGGGGCCTGGTCTAGATGGATACACAGCTGTGGGGCC

#6 CCACAGCTGTGTATCCATCTAGACCAGGCCCCAGGGGACATGGTGGTCGACG

These oligonucleotides contain complimentary sequences which upon addition to each other facilitate the annealing (base pairing) of the two individual sequences, resulting in the formation of a double stranded synthetic DNA linker (designated LINK-1) in a manner indicated below:

```
  1   5    10         20         30         40         50         60
  |   |    |          |          |          |          |          |
#5GATTCCGTCGACCACCATGTCCCCTGGGGCCTGGTCTAGATGGATACACAGCTGTGGGGCC
           GCAGCTGGTGGTACAGGGGACCCCGGACCAGATCTACCTATGTGTCGACACC #6
```

This DNA linker (LINK-1) contains recognition sequences of restriction endonucleases needed to facilitate subsequent manipulations required to construct the murine/human expression plasmid, as well as sequences required for maximal expression of heterologous sequences in mammalian cell expression systems. More specifically (referring to the sequence numbering of oligonucleotide #5/LINK-1): nucleotides #1-#11 comprise recognition sequences for the restriction endonucleases BamH I and Sal I, nucleotides #11-#15 allow for maximal expression of heterologuos sequences in mammallian cell expression systems, nucleotides #16-#31 correspond to nucleotides #610-#625 of the murine BMP-9 sequence (SEQ ID NO:1), nucleotides #32-#33 are inserted to facilitate efficient restriction digestion of two adjacent restriction endonuclease sites (EcoO109 I and Xba I), nucleotides #34-#60 correspond to nucleotides #1515-#1541 of the murine BMP-9 sequence (SEQ ID NO:1) except that nucleotide #58 of synthetic oligonucloetide #5 is a G rather than the A which appears at position #1539 of SEQ ID NO:1 (This nucleotide conversion results in the creation of an Apa I restriction

endonuclease recognition sequence, without altering the amino acid sequence it is intended to encode, to facilitate further manipulations of the murine/human hybrid expression plasmid. LINK-1 (the double stranded product of the annealing of oligonucleotides #5 and #6) is subcloned into the plasmid vector pGEM-7Zf(+) which has been digested with the restriction endonucleases Apa I and BamH I. This results in a plasmid in which the sequences normally present between the Apa I and BamH I sites of the pGEM-7Zf(+) plasmid polylinker are replaced with the sequences of LINK-1 described above. The resulting plasmid clone is designated pBMP-9link.

pBMP-9link is digested with the restriction endonucleases BamH I and Xba I resulting in the removal nucleotides #1-#34 of LINK-1 (refer to the numbering of oligo #5). Clone ML14a, which contains an insert comprising the sequence set forth in SEQ ID NO:1, is also digested with the restriction endonucleases BamH I and Xba I resulting in the removal of sequences comprising nucleotides #1-#1515 of SEQUENCE ID NO:1 (murine BMP-9). This BamH I/Xba I fragment of mouse BMP-9 is isolated from the remainder of the ML14a plasmid clone and subcloned into the BamH I/Xba I sites generated by the removal of the synthetic linker sequences described above. The resulting clone is designated p302.

The p302 clone is digested with the restriction endonuclease EcoO109 I resulting in the excision of nucleotides corresponding to nucleotides #621-#1515 of the murine BMP-9

sequence (SEQ ID NO:1) and nucleotides #35-#59 of LINK-1 (refer to numbering of oligonucleotide #5). It should be noted that the Apa I restriction site created in LINK-1 by the A to G conversion described above is a subset of the recognition sequence of EcoO109 I, therefore digestion of p302 with EcoO109 I cleaves at the Apa I site as well as the naturally occuring murine EcoO109 I (location #619-#625 of SEQ ID NO:1) resulting in the excision of a 920 bp EcoO109 I/EcoO109 I (Apa I) fragment comprising the sequences described above. This 920 EcoO109 I/EcoO109 I (Apa I) fragment is isolated from the remainder of the p302 plasmid clone and subcloned into clone pBMP-9link which has been similarly digested with EcoO109 I. It should be noted that the nucleotides GG (#32-#33 of oligonucleotide #5) originally designed to facilitate a more complete digestion of the two adjacent restriction sites EcoO109 I and Xba I of LINK-1, which is now a part of pBMP-9link (described above), results in the creation of Dcm methylation recognition sequence. The restriction nuclease EcoO109 I is sensitive to Dcm methylation and therefore cleavage of this sequence (nucleotides #25-#31 of oligonucleotide #5/LINK-1) by the restriction endonuclease EcoO109 I is prevented at this site. Therefore the plasmid clone pBMP-9link is cleaved at the Apa I site but not at the EcoO109 I site upon digestion with the restriction endonuclease EcoO109 I as described above, preventing the intended removal of the sequences between the EcoO109 I and Xba I site of LINK-1

(#32-#55 defined by the numbering of oligonucleotide #5). This results in the insertion of the 920 bp Eco0109 I/Apa I fragment at the Eco0109 I (Apa I) site of pBMP-9link. The resulting clone is designated p318.

Clone p318 is digested with the restriction endonucleases Sal I and Apa I, resulting in the excision of sequences comprising nucleotides #6-#56 of LINK-1 (refer to oligo #5 for location), nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1), and nucleotides #35-#60 of LINK-1 (refer to oligo #5 for location). The resulting 972 bp Sal I/Apa I fragment described above is isolated from the remainder of the p318 plasmid clone and will be utilized in subsequent manipulations.

The clone phBMP9mex-1 (described above), which contains DNA sequences which encode the entire mature region and portions of the propeptide of the human BMP-9 protein, is digested with the restriction endonucleases Apa I and EcoR I. This results in the excision of a 374 bp fragment comprising nucleotides #105-#470 of the human BMP-9 sequence (SEQ ID NO:8) and the additional nucleotides of oligonucleotide primers #3 and #4 which contain the recognition sequences for the restriction endonucleases Apa I and EcoR I. This 374 bp Apa I/EcoR I fragment is combined with the 972 bp Sal I/Apa I fragment from p138 (isolation described above) and ligated to the mammalian cell expression plasmid pED6 (a derivative of pEMC2β1) which has been digested with Sal I and EcoR I. The resulting clone is designated p324.

The clone ML14a (murine BMP-9) is digested with EcoO109 I and Xba I to generate a fragment comprising nucleotides #621-#1515 of SEQ ID NO:1.

The following oligonucleotides are synthesized on an automated DNA synthesizer and combined such that their complimentary sequences can base pair (anneal) with each other to generate a double stranded synthetic DNA linker designated LINK-2:

```
#7 TCGACCACCATGTCCCCTGG
#8 GCCCCAGGGGACATGGTGG
```

This double stranded synthetic DNA linker (LINK-2) anneals in such a way that it generates single stranded ends which are compatible to DNA fragments digested with Sal I (one end) or EcoO109 I (the other end) as indicated below:

```
#7 TCGACCACCATGTCCCCTGG
        GGTGGTACAGGGGACCCCG #8
```

This LINK-2 synthetic DNA linker is ligated to the 895 bp EcoO109 I/Xba I fragment comprising nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1) described above. This results in a 915 bp Sal I/Xba I fragment.

The clone p324 is digested with Sal I/Xba I to remove sequences comprising nucleotides #6-#56 of LINK-1 (refer to oligo #5 for location) and nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1). The sequences comprising nucleotides #35-#60 of LINK-1 (refer to oligo #5 for location) and the sequences comprising the 374 bp Apa I/EcoR I fragment (human BMP-9 sequences) derived from phBMP9mex-1 remain attached to

the pED6 backbone. The 915 bp Sal I/Xba I fragment comprising LINK-2 sequences and nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1) is ligated into the p324 clone from which the Sal I to Xba I sequences described above have been removed.

The resulting plasmid is designated BMP9fusion and comprises LINK-2, nucleotides #621-#1551 of murine BMP-9 (SEQ ID NO:1), nucleotides #35-#59 of LINK-1 (refer to the numbering of oligonucleotide #5), and the 374 bp Apa I/EcoR I fragment (human BMP-9) derived from clone pBMP9mex-1 (described above) inserted between the Sal I and EcoR I sites of the mammalian cell expression vector pED6.

BMP9 fusion is transfected into CHO cells using standard techniques known to those having ordinary skill in the art to create stable cell lines capable of expressing human BMP-9 protein. The cell lines are cultured under suitable culture conditions and the BMP-9 protein is isolated and purified from the culture medium.

EXAMPLE V

Biological Activity of Expressed BMP-9

To measure the biological activity of the expressed BMP-9 proteins obtained in Example IV above, the proteins are recovered from the cell culture and purified by isolating the BMP-9 proteins from other proteinaceous materials with which they are co-produced as well as from other contaminants. The purified protein may be assayed in accordance with the rat bone

formation assay described in Example III.

Purification is carried out using standard techniques known to those skilled in the art. It is contemplated, as with other BMP proteins, that purification may include the use of Heparin sepharose.

Protein analysis is conducted using standard techniques such as SDS-PAGE acrylamide [U.K. Laemmli, Nature 227:680 (1970)] stained with silver [R.R. Oakley, et al. Anal. Biochem. 105:361 (1980)] and by immunoblot [H. Towbin, et al. Proc. Natl. Acad. Sci. USA 76:4350 (1979)]

The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are believed to be encompassed within the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: BMP-9 COMPOSITIONS

(iii) NUMBER OF SEQUENCES: 9

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(F) ZIP: 02140

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2447 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus
(B) STRAIN: C57B46xCBA
(F) TISSUE TYPE: liver

```
    (vii) IMMEDIATE SOURCE:
          (A) LIBRARY: Mouse liver cDNA
          (B) CLONE: ML14A

   (viii) POSITION IN GENOME:
          (C) UNITS: bp

     (ix) FEATURE:
          (A) NAME/KEY: mat_peptide
          (B) LOCATION: 1564..1893

     (ix) FEATURE:
          (A) NAME/KEY: CDS
          (B) LOCATION: 610..1896

     (ix) FEATURE:
          (A) NAME/KEY: mRNA
          (B) LOCATION: 1..2447

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CATTAATAAA TATTAAGTAT TGGAATTAGT GAAATTGGAG TTCCTTGTGG AAGGAAGTGG      60

GCAAGTGAGC TTTTTAGTTT GTGTCGGAAG CCTGTAATTA CGGCTCCAGC TCATAGTGGA     120

ATGGCTATAC TTAGATTTAT GGATAGTTGG GTAGTAGGTG TAAATGTATG TGGTAAAAGG     180

CCTAGGAGAT TTGTTGATCC AATAAATATG ATTAGGGAAA CAATTATTAG GGTTCATGTT     240

CGTCCTTTTG GTGTGTGGAT TAGCATTATT TGTTTGATAA TAAGTTTAAC TAGTCAGTGT     300

TGGAAAGAAT GGAGACGGTT GTTGATTAGG CGTTTTGAGG ATGGGAATAG GATTGAAGGA     360

AATATAATGA TGGCTACAAC GATTGGGAAT CCTATTATTG TTGGGGTAAT GAATGAGGCA     420

AATAGATTTT CGTTCATTTT AATTCTCAAG GGGTTTTTAC TTTTATGTTT GTTAGTGATA     480

TTGGTGAGTA GGCCAAGGGT TAATAGTGTA ATTGAATTAT AGTGAAATCA TATTACTAGA     540

CCTGATGTTA GAAGGAGGGC TGAAAAGGCT CCTTCCCTCC CAGGACAAAA CCGGAGCAGG     600

GCCACCCGG ATG TCC CCT GGG GCC TTC CGG GTG GCC CTG CTC CCG CTG         648
          Met Ser Pro Gly Ala Phe Arg Val Ala Leu Leu Pro Leu
          -318        -315                    -310

TTC CTG CTG GTC TGT GTC ACA CAG CAG AAG CCG CTG CAG AAC TGG GAA       696
Phe Leu Leu Val Cys Val Thr Gln Gln Lys Pro Leu Gln Asn Trp Glu
-305                -300                -295                -290

CAA GCA TCC CCT GGG GAA AAT GCC CAC AGC TCC CTG GGA TTG TCT GGA       744
Gln Ala Ser Pro Gly Glu Asn Ala His Ser Ser Leu Gly Leu Ser Gly
                -285                -280                -275

GCT GGA GAG GAG GGT GTC TTT GAC CTG CAG ATG TTC CTG GAG AAC ATG       792
Ala Gly Glu Glu Gly Val Phe Asp Leu Gln Met Phe Leu Glu Asn Met
            -270                -265                -260

AAG GTG GAT TTC CTA CGC AGC CTT AAC CTC AGC GGC ATT CCC TCC CAG       840
Lys Val Asp Phe Leu Arg Ser Leu Asn Leu Ser Gly Ile Pro Ser Gln
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 151 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
 *  Thr Arg Glu Cys Ser Arg Ser Cys Pro Arg Thr Ala Pro Gln Arg
-41 -40                 -35                 -30

Gln Val Arg Ala Val Thr Arg Arg Thr Arg Met Ala His Val Ala Ala
-25                 -20                 -15                 -10

Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala Gly Ser His
                 -5                   1               5

Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile Gly Trp Asp
         10                  15                  20

Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu Cys Lys Gly
     25                  30                  35

Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr Lys His Ala
 40                  45                  50                  55

Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys Val Gly Lys
                 60                  65                  70

Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val Leu Tyr Lys
             75                  80                  85

Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu Gly Met Ser
         90                  95                 100

Val Ala Glu Cys Gly Cys Arg
    105                 110
```

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 1..470

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..456

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 124..453

(ix) FEATURE:
(A) NAME/KEY: mRNA
(B) LOCATION: 1..470

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
TGA ACA AGA GAG TGC TCA AGA AGC TGT CCA AGG ACG GCT CCA CAG AGG      48
 *  Thr Arg Glu Cys Ser Arg Ser Cys Pro Arg Thr Ala Pro Gln Arg
-41 -40                 -35                 -30

CAG GTG AGA GCA GTC ACG AGG AGG ACA CGG ATG GCG CAC GTG GCT GCG      96
Gln Val Arg Ala Val Thr Arg Arg Thr Arg Met Ala His Val Ala Ala
-25                 -20                 -15                 -10

GGG TCG ACT TTA GCC AGG CGG AAA AGG AGC GCC GGG GCT GGC AGC CAC     144
Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala Gly Ser His
                 -5                   1               5

TGT CAA AAG ACC TCC CTG CGG GTA AAC TTC GAG GAC ATC GGC TGG GAC     192
Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile Gly Trp Asp
         10                  15                  20

AGC TGG ATC ATT GCA CCC AAG GAG TAT GAA GCC TAC GAG TGT AAG GGC     240
Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu Cys Lys Gly
     25                  30                  35

GGC TGC TTC TTC CCC TTG GCT GAC GAT GTG ACG CCG ACG AAA CAC GCT     288
Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr Lys His Ala
 40                  45                  50                  55

ATC GTG CAG ACC CTG GTG CAT CTC AAG TTC CCC ACA AAG GTG GGC AAG     336
Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys Val Gly Lys
                 60                  65                  70

GCC TGC TGT GTG CCC ACC AAA CTG AGC CCC ATC TCC GTC CTC TAC AAG     384
Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val Leu Tyr Lys
             75                  80                  85

GAT GAC ATG GGG GTG CCC ACC CTC AAG TAC CAT TAC GAG GGC ATG AGC     432
Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu Gly Met Ser
         90                  95                 100

GTG GCA GAG TGT GGG TGC AGG TAGTATCTGC CTGCGGG                      470
Val Ala Glu Cys Gly Cys Arg
    105                 110
```

CATGGGCAGC TCGAG

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTGCAGGCGA GCCTGAATTC CTCGAGCCAT CATG 34

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGAGGTTAAA AAACGTCTAG GCCCCCCGAA CCACGGGGAC GTGGTTTTCC TTTGAAAAAC 60

ACGATTGC 68

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 470 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(H) CELL LINE: W138 (genomic DNA)

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: human genomic library
(B) CLONE: lambda 111-1

(viii) POSITION IN GENOME:
(C) UNITS: bp

Arg Ile Asn Ile Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro
    -115   -110   -105

Gly His Leu Ile Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn
-100   -95   -90   -85

Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp
                -80   -75   -70

Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His
            -65   -60   -55

Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg
        -50   -45   -40

Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu
    -35   -30   -25

Val Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg
-20   -15   -10   -5

Arg Ala Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys
                1   5   10

Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val
        15   20   25

Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr
    30   35   40

Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
45   50   55   60

Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile
                65   70   75

Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
            80   85   90

Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met
        95   100   105

Val Val Glu Gly Cys Gly Cys Arg
    110   115

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG ATATACACAC      1666
Cys Gly Cys Arg
        115

CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC      1726

ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA      1786

ATCCCTAAAC ATTCACCTTG ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT      1846

TGATCATATA TTTTGACAAA ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG      1906

AGTCATTATT TTAAAAAAAA AAAAAAAACT CTAGAGTCGA CGGAATTC                 1954
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ile Pro Gly Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val
-292    -290                -285                -280

Leu Leu Gly Gly Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys
    -275                -270                -265

Lys Lys Val Ala Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly
-260                -255                -250                -245

Gln Ser His Glu Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met
            -240                -235                -230

Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro
        -225                -220                -215

Asp Tyr Met Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu
    -210                -205                -200

Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser
    -195                -190                -185

Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn
-180                -175                -170                -165

Ile Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu
            -160                -155                -150

Ser Ser Ile Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu
        -145                -140                -135

Phe Arg Glu Gln Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His
        -130                -125                -120
```

```
GTG GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT     942
Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile
            -125                -120                -115

TAT GAG GTT ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC     990
Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile
        -110                -105                -100

ACA CGA CTA CTG GAC ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG    1038
Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn Val Thr Arg Trp
    -95                 -90                 -85

GAA ACT TTT GAT GTG AGC CCT GCG GTC CTT CGC TGG ACC CGG GAG AAG    1086
Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp Thr Arg Glu Lys
-80                 -75                 -70                 -65

CAG CCA AAC TAT GGG CTA GCC ATT GAG GTG ACT CAC CTC CAT CAG ACT    1134
Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His Leu His Gln Thr
                -60                 -55                 -50

CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC CGA TCG TTA CCT CAA    1182
Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg Ser Leu Pro Gln
            -45                 -40                 -35

GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC ACC TTT GGC    1230
Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val Thr Phe Gly
        -30                 -25                 -20

CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG CGT    1278
His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys Arg
    -15                 -10                 -5

AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC    1326
Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys
1               5                   10                  15

CGG CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC    1374
Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp
            20                  25                  30

TGG ATT GTG GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC    1422
Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp
        35                  40                  45

TGC CCC TTT CCA CTG GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT    1470
Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala Ile
    50                  55                  60

GTG CAG ACC CTG GTC AAT TCT GTC AAT TCC AGT ATC CCC AAA GCC TGT    1518
Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala Cys
65                  70                  75                  80

TGT GTG CCC ACT GAA CTG AGT GCC ATC TCC ATG CTG TAC CTG GAT GAG    1566
Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu
                85                  90                  95

TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG ATG GTA GTA GAG GGA    1614
Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu Gly
            100                 105                 110
```

(B) LOCATION: 9..1934

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGCGC GGAGCCCGGC CCGGAAGCTA      60

GGTGAGTGTG GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG     120

AGTATCTAGC TTGTCTCCCC GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC     180

ACAGTCCCCG GCCCTCGCCC AGGTTCACTG CAACCGTTCA GAGGTCCCCA GGAGCTGCTG     240

CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC GTAGTGCCAT CCCGAGCAAC     300

GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG CTGTCAAGAA     360

TCATGGACTG TTATTATATG CCTTGTTTTC TGTCAAGACA CC ATG ATT CCT GGT        414
                                               Met Ile Pro Gly
                                               -292    -290

AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC       462
Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly
            -285                -280                -275

GCG AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC       510
Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala
        -270                -265                -260

GAG ATT CAG GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG       558
Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu
    -255                -250                -245

CTC CTG CGG GAC TTC GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC       606
Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met Phe Gly Leu Arg
-240                -235                -230                -225

CGC CGC CCG CAG CCT AGC AAG AGT GCC GTC ATT CCG GAC TAC ATG CGG       654
Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro Asp Tyr Met Arg
                -220                -215                -210

GAT CTT TAC CGG CTT CAG TCT GGG GAG GAG GAG GAA GAG CAG ATC CAC       702
Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu Glu Gln Ile His
            -205                -200                -195

AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC AGC CGG GCC AAC ACC       750
Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser Arg Ala Asn Thr
        -190                -185                -180

GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC CCA GGG ACC       798
Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile Pro Gly Thr
    -175                -170                -165

AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC CCT       846
Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile Pro
-160                -155                -150                -145

GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG       894
Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln
                -140                -135                -130
```

```
              -10                       -5                   1
Gly Ala Ser Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu
         5                  10                  15

Asp Ile Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Asp Ala
     20                  25                  30

Tyr Glu Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr
 35                  40                  45                  50

Pro Thr Lys His Ala Ile Val Gln Thr Leu Val His Leu Glu Phe Pro
                 55                  60                  65

Thr Lys Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile
             70                  75                  80

Ser Ile Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His
         85                  90                  95

Tyr Glu Gly Met Ser Val Ala Glu Cys Gly Cys Arg
    100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1954 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(G) CELL TYPE: Osteosarcoma Cell Line
(H) CELL LINE: U-2OS

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: U2OS cDNA in Lambda gt10
(B) CLONE: Lambda U2OS-3

(viii) POSITION IN GENOME:
(C) UNITS: bp

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 403..1629

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1279..1626

(ix) FEATURE:
(A) NAME/KEY: mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Pro Gly Ala Phe Arg Val Ala Leu Leu Pro Leu Phe Leu Leu
-318 -315 -310 -305

Val Cys Val Thr Gln Gln Lys Pro Leu Gln Asn Trp Glu Gln Ala Ser
-300 -295 -290

Pro Gly Glu Asn Ala His Ser Ser Leu Gly Leu Ser Gly Ala Gly Glu
-285 -280 -275

Glu Gly Val Phe Asp Leu Gln Met Phe Leu Glu Asn Met Lys Val Asp
-270 -265 -260 -255

Phe Leu Arg Ser Leu Asn Leu Ser Gly Ile Pro Ser Gln Asp Lys Thr
-250 -245 -240

Arg Ala Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr
-235 -230 -225

Thr Asp Lys Ser Ser Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser
-220 -215 -210

Val Glu Asp Ala Ile Ser Thr Ala Ala Thr Glu Asp Phe Pro Phe Gln
-205 -200 -195

Lys His Ile Leu Ile Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile
-190 -185 -180 -175

Thr Arg Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn Asp Val Asp
-170 -165 -160

Ser Thr His Gly Leu Glu Gly Ser Met Val Val Tyr Asp Val Leu Glu
-155 -150 -145

Asp Ser Glu Thr Trp Asp Gln Ala Thr Gly Thr Lys Thr Phe Leu Val
-140 -135 -130

Ser Gln Asp Ile Arg Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser
-125 -120 -115

Ala Val Lys Arg Trp Val Arg Ala Asp Ser Thr Thr Asn Lys Asn Lys
-110 -105 -100 -95

Leu Glu Val Thr Val Gln Ser His Arg Glu Ser Cys Asp Thr Leu Asp
-90 -85 -80

Ile Ser Val Pro Pro Gly Ser Lys Asn Leu Pro Phe Phe Val Val Phe
-75 -70 -65

Ser Asn Asp Arg Ser Asn Gly Thr Lys Glu Thr Arg Leu Glu Leu Lys
-60 -55 -50

Glu Met Ile Gly His Glu Gln Glu Thr Met Leu Val Lys Thr Ala Lys
-45 -40 -35

Asn Ala Tyr Gln Val Ala Gly Glu Ser Gln Glu Glu Glu Gly Leu Asp
-30 -25 -20 -15

Gly Tyr Thr Ala Val Gly Pro Leu Leu Ala Arg Arg Lys Arg Ser Thr

```
    -15                 -10                  -5

AGG AGC ACC GGA GCC AGC AGC CAC TGC CAG AAG ACT TCT CTC AGG GTG        1608
Arg Ser Thr Gly Ala Ser Ser His Cys Gln Lys Thr Ser Leu Arg Val
     1               5                  10                  15

AAC TTT GAG GAC ATC GGC TGG GAC AGC TGG ATC ATT GCA CCC AAG GAA        1656
Asn Phe Glu Asp Ile Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu
                 20                  25                  30

TAT GAC GCC TAT GAG TGT AAA GGG GGT TGC TTC TTC CCA TTG GCT GAT        1704
Tyr Asp Ala Tyr Glu Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp
             35                  40                  45

GAC GTG ACA CCC ACC AAA CAT GCC ATC GTG CAG ACC CTG GTG CAT CTC        1752
Asp Val Thr Pro Thr Lys His Ala Ile Val Gln Thr Leu Val His Leu
         50                  55                  60

GAG TTC CCC ACA AAG GTG GGC AAA GCC TGC TGC GTT CCC ACC AAA CTG        1800
Glu Phe Pro Thr Lys Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu
     65                  70                  75

AGT CCC ATC TCC ATC CTC TAC AAG GAT GAC ATG GGG GTG CCA ACC CTC        1848
Ser Pro Ile Ser Ile Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu
 80                  85                  90                  95

AAG TAC CAC TAT GAG GGG ATG AGT GTG GCT GAG TGT GGG TGT AGG TAGTCCCTGC   1903
Lys Tyr His Tyr Glu Gly Met Ser Val Ala Glu Cys Gly Cys Arg
                100                 105                 110

AGCCACCCAG GGTGGGGATA CAGGACATGG AAGAGGTTCT GGTACGGTCC TGCATCCTCC      1963

TGCGCATGGT ATGCCTAAGT TGATCAGAAA CCATCCTTGA GAAGAAAAGG AGTTAGTTGC      2023

CCTTCTTGTG TCTGGTGGGT CCCTCTGCTG AAGTGACAAT GACTGGGGTA TGCGGGCCTG      2083

TGGGCAGAGC AGGAGACCCT GGAAGGGTTA GTGGGTAGAA AGATGTCAAA AAGGAAGCTG      2143

TGGGTAGATG ACCTGCACTC CAGTGATTAG AAGTCCAGCC TTACCTGTGA GAGAGCTCCT      2203

GGCATCTAAG AGAACTCTGC TTCCTCATCA TCCCCACCGA CTTGTTCTTC CTTGGGAGTG      2263

TGTCCTCAGG GAGAACAGCA TTGCTGTTCC TGTGCCTCAA GCTCCCAGCT GACTCTCCTG      2323

TGGCTCATAG GACTGAATGG GGTGAGGAAG AGCCTGATGC CCTCTGGCAA TCAGAGCCCG      2383

AAGGACTTCA AAACATCTGG ACAACTCTCA TTGACTGATG CTCCAACATA ATTTTTAAAA      2443

AGAG                                                                   2447
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 428 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

-255 -250 -245

GAC AAA ACC AGA GCG GAG CCA CCC CAG TAC ATG ATC GAC TTG TAC AAC 888
Asp Lys Thr Arg Ala Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn
-240 -235 -230

AGA TAC ACA ACG GAC AAA TCG TCT ACG CCT GCC TCC AAC ATC GTG CGG 936
Arg Tyr Thr Thr Asp Lys Ser Ser Thr Pro Ala Ser Asn Ile Val Arg
-225 -220 -215 -210

AGC TTC AGC GTG GAA GAT GCT ATA TCG ACA GCT GCC ACG GAG GAC TTC 984
Ser Phe Ser Val Glu Asp Ala Ile Ser Thr Ala Ala Thr Glu Asp Phe
-205 -200 -195

CCC TTT CAG AAG CAC ATC CTG ATC TTC AAC ATC TCC ATC CCG AGG CAC 1032
Pro Phe Gln Lys His Ile Leu Ile Phe Asn Ile Ser Ile Pro Arg His
-190 -185 -180

GAG CAG ATC ACC AGG GCT GAG CTC CGA CTC TAT GTC TCC TGC CAA AAT 1080
Glu Gln Ile Thr Arg Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn
-175 -170 -165

GAT GTG GAC TCC ACT CAT GGG CTG GAA GGA AGC ATG GTC GTT TAT GAT 1128
Asp Val Asp Ser Thr His Gly Leu Glu Gly Ser Met Val Val Tyr Asp
-160 -155 -150

GTT CTG GAG GAC AGT GAG ACT TGG GAC CAG GCC ACG GGG ACC AAG ACC 1176
Val Leu Glu Asp Ser Glu Thr Trp Asp Gln Ala Thr Gly Thr Lys Thr
-145 -140 -135 -130

TTC TTG GTA TCC CAG GAC ATT CGG GAC GAA GGA TGG GAG ACT TTA GAA 1224
Phe Leu Val Ser Gln Asp Ile Arg Asp Glu Gly Trp Glu Thr Leu Glu
-125 -120 -115

GTA TCG AGT GCC GTG AAG CGG TGG GTC AGG GCA GAC TCC ACA ACA AAC 1272
Val Ser Ser Ala Val Lys Arg Trp Val Arg Ala Asp Ser Thr Thr Asn
-110 -105 -100

AAA AAT AAG CTC GAG GTG ACA GTG CAG AGC CAC AGG GAG AGC TGT GAC 1320
Lys Asn Lys Leu Glu Val Thr Val Gln Ser His Arg Glu Ser Cys Asp
-95 -90 -85

ACA CTG GAC ATC AGT GTC CCT CCA GGT TCC AAA AAC CTG CCC TTC TTT 1368
Thr Leu Asp Ile Ser Val Pro Pro Gly Ser Lys Asn Leu Pro Phe Phe
-80 -75 -70

GTT GTC TTC TCC AAT GAC CGC AGC AAT GGG ACC AAG GAG ACC AGA CTG 1416
Val Val Phe Ser Asn Asp Arg Ser Asn Gly Thr Lys Glu Thr Arg Leu
-65 -60 -55 -50

GAG CTG AAG GAG ATG ATC GGC CAT GAG CAG GAG ACC ATG CTT GTG AAG 1464
Glu Leu Lys Glu Met Ile Gly His Glu Gln Glu Thr Met Leu Val Lys
-45 -40 -35

ACA GCC AAA AAT GCT TAC CAG GTG GCA GGT GAG AGC CAA GAG GAG GAG 1512
Thr Ala Lys Asn Ala Tyr Gln Val Ala Gly Glu Ser Gln Glu Glu Glu
-30 -25 -20

GGT CTA GAT GGA TAC ACA GCT GTG GGA CCA CTT TTA GCT AGA AGG AAG 1560
Gly Leu Asp Gly Tyr Thr Ala Val Gly Pro Leu Leu Ala Arg Arg Lys

What is claimed is:

1. A BMP-9 polypeptide comprising the amino acid sequence from amino acid #8 - 110 as set forth in FIG. 3 (SEQ ID NO: 9).

2. A BMP-9 polypeptide comprising the amino acid sequence from amino acid #1 - 110 as set forth in FIG. 3 (SEQ ID NO: 9).

3. A BMP-9 polypeptide of claim 1 wherein said polypeptide is a dimer wherein each subunit comprises at least the amino acid sequence from amino acid #8 - 110 of FIG. 3 (SEQ ID NO: 9).

4. A BMP-9 polypeptide of claim 2 wherein said polypeptide is a dimer wherein each subunit comprises at least the amino acid sequence from amino acid #1-110 of FIG. 3. (SEQ ID NO: 9).

5. A purified BMP-9 protein produced by the steps of

(a) culturing a cell transformed with a cDNA comprising the nucleotide sequence from nucleotide #124 to #453 as shown in FIG. 3 (SEQ ID NO: 8); and

(b) recovering and purifying from said culture medium a protein comprising the amino acid sequence from amino acid #1 to amino acid #110 as shown in FIG. 3 (SEQ ID NO: 9).

6. A purified BMP-9 protein produced by the steps of

(a) culturing a cell transformed with a cDNA comprising

the nucleotide sequence from nucleotide #124 to #453 as shown in FIG. 3 (SEQ ID NO: 8); and

(b) recovering form said culture medium a protein comprising an amino acid sequence from amino acid #8 to amino acid #110 as shown in Figure 3 (SEQ ID NO: 9).

7. A BMP-9 protein characterized by the ability to induce the formation of cartilage and/or bone.

8. A DNA sequence encoding a BMP-9 protein.

9. The DNA sequence of claim 8 wherein said DNA comprises

(a) nucleotide 124 to 453 (SEQ ID NO: 8); and

(b) sequences which hybridize thereto under stringent hybridization conditions and exhibit the ability to form cartilage and/or bone.

10. The DNA sequence of claim 8 wherein said DNA comprises

(a) nucleotide 145 to 453 (SEQ ID NO: 8); and

(b) sequences which hybridize thereto under stringent hybridization conditions and exhibit the ability to form cartilage and/or bone.

11. A host cell transformed with a DNA sequence encoding BMP-8.

12. A method for producing a purified BMP-9 protein said method comprising the steps of

(a) culturing a cell transformed with a cDNA comprising the nucleotide sequence encoding a BMP-9 protein; and

(b) recovering and purifying said BMP-9 protein from the culture medium.

13. A pharmaceutical composition comprising an effective amount of a BMP-9 protein in admixture with a pharmaceutically acceptable vehicle.

14. A composition of claim 13 further comprising a matrix for supporting said composition and providing a surface for bone and/or cartilage growth.

15. The composition of claim 14 wherein said matrix comprises a material selected from the group consisting of hydroxyapatite, collagen, polylactic acid and tricalcium phosphate.

16. A method for inducing bone and/or cartilage formation in a patient in need of same comprising administering to said patient an effective amount of the composition of claim 13.

17. A pharmaceutical composition for wound healing and tissue repair said composition comprising an effective amount of the

protein of a BMP-9 protein in a pharmaceutically acceptable vehicle.

18. A method for treating wounds and/or tissue repair in a patient in need of same comprising administering to said patient an effective amount of the composition of claim 17.

Figure 1A

```
        10         20         30         40         50         60         70
CATTAATAAA TATTAAGTAT TGGAATTAGT GAAATTGGAG TTCCTTGTGG AAGGAAGTGG GCAAGTGAGC

        80         90        100        110        120        130        140
TTTTTAGTTT GTGTCGGAAG CCTGTAATTA CGGCTCCAGC TCATAGTGGA ATGGCTATAC TTAGATTTAT
       150        160        170        180        190        200        210
GGATAGTTGG GTAGTAGGTG TAAATGTATG TGGTAAAAGG CCTAGGAGAT TTGTTGATCC AATAAATATG
       220        230        240        250        260        270        280
ATTAGGGAAA CAATTATTAG GGTTCATGTT CGTCCTTTTG GTGTGTGGAT TAGCATTATT TGTTTGATAA
       290        300        310        320        330        340        350
TAAGTTTAAC TAGTCAGTGT TGGAAAGAAT GGAGACGGTT GTTGATTAGG CGTTTTGAGG ATGGGAATAG
       360        370        380        390        400        410        420
GATTGAAGGA AATATAATGA TGGCTACAAC GATTGGGAAT CCTATTATTG TTGGGGTAAT GAATGAGGCA
       430        440        450        460        470        480        490
AATAGATTTT CGTTCATTTT AATTCTCAAG GGGTTTTTAC TTTTATGTTT GTTAGTGATA TTGGTGAGTA
       500        510        520        530        540        550        560
GGCCAAGGGT TAATAGTGTA ATTGAATTAT AGTGAAATCA TATTACTAGA CCTGATGTTA GAAGGAGGGC
        570        580        590        600        609       618
 TGAAAAGGCT CCTTCCCTCC CAGGACAAAA CCGGAGCAGG GCCACCCGG >ATG TCC CCT GGG
                                                        M   S   P   G

     627         636         645         654         663         672
GCC TTC CGG GTG GCC CTG CTC CCG CTG TTC CTG CTG GTC TGT GTC ACA CAG CAG
A   F   R   V   A   L   L   P   L   F   L   L   V   C   V   T   Q   Q

     681         690         699         708         717         726
AAG CCG CTG CAG AAC TGG GAA CAA GCA TCC CCT GGG GAA AAT GCC CAC AGC TCC
K   P   L   Q   N   W   E   Q   A   S   P   G   E   N   A   H   S   S

     735         744         753         762         771         780
CTG GGA TTG TCT GGA GCT GGA GAG GAG GGT GTC TTT GAC CTG CAG ATG TTC CTG
L   G   L   S   G   A   G   E   E   G   V   F   D   L   Q   M   F   L

     789         798         807         816         825         834
GAG AAC ATG AAG GTG GAT TTC CTA CGC AGC CTT AAC CTC AGC GGC ATT CCC TCC
E   N   M   K   V   D   F   L   R   S   L   N   L   S   G   I   P   S
```

Figure 1B

	843			852			861			870			879			888	
CAG	GAC	AAA	ACC	AGA	GCG	GAG	CCA	CCC	CAG	TAC	ATG	ATC	GAC	TTG	TAC	AAC	AGA
Q	D	K	T	R	A	E	P	P	Q	Y	M	I	D	L	Y	N	R
	897			906			915			924			933			942	
TAC	ACA	ACG	GAC	AAA	TCG	TCT	ACG	CCT	GCC	TCC	AAC	ATC	GTG	CGG	AGC	TTC	AGC
Y	T	T	D	K	S	S	T	P	A	S	N	I	V	R	S	F	S
	951			960			969			978			987			996	
GTG	GAA	GAT	GCT	ATA	TCG	ACA	GCT	GCC	ACG	GAG	GAC	TTC	CCC	TTT	CAG	AAG	CAC
V	E	D	A	I	S	T	A	A	T	E	D	F	P	F	Q	K	H
	1005			1014			1023			1032			1041			1050	
ATC	CTG	ATC	TTC	AAC	ATC	TCC	ATC	CCG	AGG	CAC	GAG	CAG	ATC	ACC	AGG	GCT	GAG
I	L	I	F	N	I	S	I	P	R	H	E	Q	I	T	R	A	E
	1059			1068			1077			1086			1095			1104	
CTC	CGA	CTC	TAT	GTC	TCC	TGC	CAA	AAT	GAT	GTG	GAC	TCC	ACT	CAT	GGG	CTG	GAA
L	R	L	Y	V	S	C	Q	N	D	V	D	S	T	H	G	L	E
	1113			1122			1131			1140			1149			1158	
GGA	AGC	ATG	GTC	GTT	TAT	GAT	GTT	CTG	GAG	GAC	AGT	GAG	ACT	TGG	GAC	CAG	GCC
G	S	M	V	V	Y	D	V	L	E	D	S	E	T	W	D	Q	A
	1167			1176			1185			1194			1203			1212	
ACG	GGG	ACC	AAG	ACC	TTC	TTG	GTA	TCC	CAG	GAC	ATT	CGG	GAC	GAA	GGA	TGG	GAG
T	G	T	K	T	F	L	V	S	Q	D	I	R	D	E	G	W	E
	1221			1230			1239			1248			1257			1266	
ACT	TTA	GAA	GTA	TCG	AGT	GCC	GTG	AAG	CGG	TGG	GTC	AGG	GCA	GAC	TCC	ACA	ACA
T	L	E	V	S	S	A	V	K	R	W	V	R	A	D	S	T	T
	1275			1284			1293			1302			1311			1320	
AAC	AAA	AAT	AAG	CTC	GAG	GTG	ACA	GTG	CAG	AGC	CAC	AGG	GAG	AGC	TGT	GAC	ACA
N	K	N	K	L	E	V	T	V	Q	S	H	R	E	S	C	D	T
	1329			1338			1347			1356			1365			1374	
CTG	GAC	ATC	AGT	GTC	CCT	CCA	GGT	TCC	AAA	AAC	CTG	CCC	TTC	TTT	GTT	GTC	TTC
L	D	I	S	V	P	P	G	S	K	N	L	P	F	F	V	V	F

Figure 1C

1383 1392 1401 1410 1419 1428
TCC AAT GAC CGC AGC AAT GGG ACC AAG GAG ACC AGA CTG GAG CTG AAG GAG ATG
S N D R S N G T K E T R L E L K E M

1437 1446 1455 1464 1473 1482
ATC GGC CAT GAG CAG GAG ACC ATG CTT GTG AAG ACA GCC AAA AAT GCT TAC CAG
I G H E Q E T M L V K T A K N A Y Q

1491 1500 1509 1518 1527 1536
GTG GCA GGT GAG AGC CAA GAG GAG GAG GGT CTA GAT GGA TAC ACA GCT GTG GGA
V A G E S Q E E E G L D G Y T A V G

1545 1554 1563 1572 1581 1590
CCA CTT TTA GCT AGA AGG AAG AGG AGC ACC GGA GCC AGC AGC CAC TGC CAG AAG
P L L A R R K R S T G A S S H C Q K
(319) (326)

1599 1608 1617 1626 1635 1644
ACT TCT CTC AGG GTG AAC TTT GAG GAC ATC GGC TGG GAC AGC TGG ATC ATT GCA
T S L R V N F E D I G W D S W I I A

1653 1662 1671 1680 1689 1698
CCC AAG GAA TAT GAC GCC TAT GAG TGT AAA GGG GGT TGC TTC TTC CCA TTG GCT
P K E Y D A Y E C K G G C F F P L A

1707 1716 1725 1734 1743 1752
GAT GAC GTG ACA CCC ACC AAA CAT GCC ATC GTG CAG ACC CTG GTG CAT CTC GAG
D D V T P T K H A I V Q T L V H L E

1761 1770 1779 1788 1797 1806
TTC CCC ACA AAG GTG GGC AAA GCC TGC TGC GTT CCC ACC AAA CTG AGT CCC ATC
F P T K V G K A C C V P T K L S P I

1815 1824 1833 1842 1851 1860
TCC ATC CTC TAC AAG GAT GAC ATG GGG GTG CCA ACC CTC AAG TAC CAC TAT GAG
S I L Y K D D M G V P T L K Y H Y E

1869 1878 1887 1903 1913 1923
GGG ATG AGT GTG GCT GAG TGT GGG TGT AGG TAGTCCCTGC AGCCACCCAG GGTGGGATA
G M S V A E C G C R
(428)

Figure 1D

```
      1933       1943       1953       1963       1973       1983       1993
CAGGACATGG AAGAGGTTCT GGTACGGTCC TGCATCCTCC TGCGCATGGT ATGCCTAAGT TGATCAGAAA
      2003       2013       2023       2033       2043       2053       2063
CCATCCTTGA GAAGAAAAGG AGTTAGTTGC CCTTCTTGTG TCTGGTGGGT CCCTCTGCTG AAGTGACAAT
      2073       2083       2093       2103       2113       2123       2133
GACTGGGGTA TGCGGGCCTG TGGGCAGAGC AGGAGACCCT GGAAGGGTTA GTGGGTAGAA AGATGTCAAA
      2143       2153       2163       2173       2183       2193       2203
AAGGAAGCTG TGGGTAGATG ACCTGCACTC CAGTGATTAG AAGTCCAGCC TTACCTGTGA GAGAGCTCCT
      2213       2223       2233       2243       2253       2263       2273
GGCATCTAAG AGAACTCTGC TTCCTCATCA TCCCCACCGA CTTGTTCTTC CTTGGGAGTG TGTCCTCAGG
      2283       2293       2303       2313       2323       2333       2343
GAGAACAGCA TTGCTGTTCC TGTGCCTCAA GCTCCCAGCT GACTCTCCTG TGGCTCATAG GACTGAATGG
      2353       2363       2373       2383       2393       2403       2413
GGTGAGGAAG AGCCTGATGC CCTCTGGCAA TCAGAGCCCG AAGGACTTCA AAACATCTGG ACAACTCTCA
      2423       2433       2443
TTGACTGATG CTCCAACATA ATTTTTAAAA AGAG
```

Figure 2

```
        10         20         30         40         50         60         70
CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGCGC GGAGCCCGGC CCGGAAGCTA GGTGAGTGTG

        80         90        100        110        120        130        140
GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG AGTATCTAGC TTGTCTCCCC

       150        160        170        180        190        200        210
GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC ACAGTCCCCG GCCCTCGCCC AGGTTCACTG

       220        230        240        250        260        270        280
CAACCGTTCA GAGGTCCCCA GGAGCTGCTG CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC

       290        300        310        320        330        340        350
GTAGTGCCAT CCCGAGCAAC GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG

       360        370        380        390        400    (1)
CTGTCAAGAA TCATGGACTG TTATTATATG CCTTGTTTTC TGTCAAGACA CC ATG ATT CCT
                                                          MET Ile Pro

    417                 432                 447                 462
GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG
Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala

            477                 492                 507
AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG
Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln

522                 537                 552                 567
GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CGG GAC TTC
Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe

        582                 597                 612                 627
GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CCG CAG CCT AGC AAG
Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys

                642                 657                 672
AGT GCC GTC ATT CCG GAC TAC ATG CGG GAT CTT TAC CGG CTT CAG TCT GGG GAG
Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu

    687                 702                 717                 732
GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC
Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala

            747                 762                 777
AGC CGG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile

792                 807                 822                 837
CCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC
Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile
```

Figure 2A

```
    852                           867                           882                           897
CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG GTG
Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val

                912                           927                           942
GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT
Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val

    957                           972                           987                           1002
ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC ACA CGA CTA CTG GAC
MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp

            1017                          1032                          1047
ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC CCT
Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro

1062                      1077                          1092                          1107
GCG GTC CTT CGC TGG ACC CGG GAG AAG CAG CCA AAC TAT GGG CTA GCC ATT GAG
Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu

        1122                          1137                          1152                          1167
GTG ACT CAC CTC CAT CAG ACT CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC
Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser

                1182                          1197                          1212
CGA TCG TTA CCT CAA GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC
Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val

    1227                          1242                          1257                          1272
ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG
Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys

            1287                          1302                          1317
CGT AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG
Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg

1332(311)                 1347                          1362                          1377
CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val

        1392                          1407                          1422                          1437
GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG
Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu

                1452                          1467                          1482
GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT
Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

    1497                          1512                          1527                          1542
GTC AAT TCC AGT ATC CCC AAA GCC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC
Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
```

Figure 2B

```
             1557                          1572                          1587
TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu

1602                    1617           (408)      1636       1646       1656
ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG
MET Val Val Glu Gly Cys Gly Cys Arg

      1666       1676       1686       1696       1706       1716       1726
ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC

      1736       1746       1756       1766       1776       1786       1796
ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC

      1806       1816       1826       1836       1846       1856       1866
ATTCACCTTG ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA TTTTGACAAA

      1876       1886       1896       1906       1916       1926       1936
ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAAA AAAAAAAACT

      1946
CTAGAGTCGA CGGAATTC
```

Figure 3

```
TGA ACA AGA GAG TGC TCA AGA AGC TGT CCA AGG ACG GCT CCA CAG AGG      48
 *  Thr Arg Glu Cys Ser Arg Ser Cys Pro Arg Thr Ala Pro Gln Arg
-41 -40                 -35                 -30

CAG GTG AGA GCA GTC ACG AGG AGG ACA CGG ATG GCG CAC GTG GCT GCG      96
Gln Val Arg Ala Val Thr Arg Arg Thr Arg Met Ala His Val Ala Ala
-25                 -20                 -15                 -10

GGG TCG ACT TTA GCC AGG CGG AAA AGG AGC GCC GGG GCT GGC AGC CAC     144
Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala Gly Ser His
                -5                   1                   5

TGT CAA AAG ACC TCC CTG CGG GTA AAC TTC GAG GAC ATC GGC TGG GAC     192
Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile Gly Trp Asp
         10                  15                  20

AGC TGG ATC ATT GCA CCC AAG GAG TAT GAA GCC TAC GAG TGT AAG GGC     240
Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu Cys Lys Gly
     25                  30                  35

GGC TGC TTC TTC CCC TTG GCT GAC GAT GTG ACG CCG ACG AAA CAC GCT     288
Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr Lys His Ala
 40                  45                  50                  55

ATC GTG CAG ACC CTG GTG CAT CTC AAG TTC CCC ACA AAG GTG GGC AAG     336
Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys Val Gly Lys
                 60                  65                  70

GCC TGC TGT GTG CCC ACC AAA CTG AGC CCC ATC TCC GTC CTC TAC AAG     384
Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val Leu Tyr Lys
             75                  80                  85

GAT GAC ATG GGG GTG CCC ACC CTC AAG TAC CAT TAC GAG GGC ATG AGC     432
Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu Gly Met Ser
         90                  95                 100

GTG GCA GAG TGT GGG TGC AGG TAGTATCTGC CTGCGGG                      470
Val Ala Glu Cys Gly Cys Arg
    105                 110
```

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/05374

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C12P21/02; A61K37/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; C12N ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	WO,A,9 011 366 (GENETICS INSTITUTE, INC.) 4 October 1990 cited in the application see the whole document ---	1-18
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 87, no. 24, December 1990, WASHINGTON US pages 9843 - 9847 CELESTE, A.J. ET AL. 'Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone' see the whole document ---	1-18

-/--

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
05 OCTOBER 1992	19. 10. 9?
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	ANDRES S.M.

2

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,A	WO,A,9 118 098 (GENETICS INSTITUTE, INC.) 28 November 1991 cited in the application see the whole document -----	1-18

INTERNATIONAL SEARCH REPORT	International application No. PCT/ US 92/ 05374

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 16, 18 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged affects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9205374 SA 61850

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 05/10/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9011366	04-10-90	US-A- 5106748	21-04-92
		AU-A- 5357790	22-10-90
		CA-A- 2030518	29-09-90
		EP-A- 0429570	05-06-91
		JP-T- 3505098	07-11-91
WO-A-9118098	28-11-91	None	

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82